REMARKS

In an Office Action mailed March 12, 2007, the Examiner in charge of the above-identified application rejected the claims under 35 U.S.C. §§ 112, 1st and 2nd paragraph and 103(a). Claims 1-16 are pending in the application; Claims 5, 6, 11, 14-16 are withdrawn from consideration as being drawn to a non-elected invention, and Claims 1-4, 7-10, 12 and 13 are rejected. Applicants respond to the Examiner's objections and rejections below. In view of the amendments noted above and the remarks presented herein, applicants respectfully request reconsideration of the merits of this application.

Claim Objections

Claims 7, 8 and 10 were objected to because of language informalities. The claim informalities are corrected herein above.

Claim Rejections - 35 USC §112, second paragraph

Claims 1, 7-10, 12 and 13 are rejected under 35 USC 112, second paragraph as indefinite. The perceived indefiniteness to Claims 1, 7, 10 and 12 is corrected herein above.

Claim Rejections - 35 USC §112, first paragraph

Claims 1-4, 7-10, 12 and 13 are rejected under 35 USC 112,first paragraph, as lacking enablement. Applicants traverse the rejection.

The Examiner asserts that the specification enables

> "targeted modifications of embryonic stem cells by electroporating copies of 1) a targeting vector comprising a foreign gene and a marker flanked by 3' and 5' homologous arms and 2) identifying cells which contain the genetic construct by detection of the marker 3) in the absence of a promoter, the construct is designed to recombine such that the marker gene is operably linked to an endogenous, tissue specific promoter 4) and further for purifying cells of a defined lineage, the marker gene comprises a promoter active in cells of a defined lineage." (See, pg. 7 of Office Action).

The Examiner asserts the specification does not reasonably enable any other embodiment. It is asserted that the scope of the invention is extremely broad in that the cells are modified by transformation with *any* foreign gene, the marker is inserted into *any* location, and the methods can be used to purify cells of *any* defined lineage. However, the Examiner asserts

that only a single location is described in the specification from which the cell lineage can be assayed (i.e., the Oct4 region where a promoterless construct is inserted into the 3'UTR region. (See pg. 9, 1st para. of the present Office Action). The Examiner goes on to assert that the language "obtaining copies of a genetic construct which includes a foreign gene and which has regions at either of its ends homologous with a pair of selected regions in the genome" in Claims 1 and 7 is unclear. Applicants disagree with the Examiner's assessment of the claims.

However, for clarification purposes, applicants amend independent Claims 1, 7 and 12. Specifically, Claims 1 and 7 are amended to affirmatively recite that the targeting vector includes 5' and 3' arms flanking an insert that is homologous to a genomic region flanking a site in the genome of ES cells selected for such insertion to allow for homologous recombination. Claims 1 and 7 are amended to recite that the construct includes a marker gene for cellular identification. Accordingly, Claim 2 is canceled and Claims 3 and 4 are amended to refer to Claim 1. Also, Claim 12 is amended to correct its dependency and to clarify that the genes identified in the "analysis step" are used to direct differentiation of human ES cells into a defined lineage, which is then purified based on gene expression.

Furthermore, new Claims 17 and 18 are added in an effort to more specifically define the claimed embodiments and to identify as well as establish allowable subject matter. These new claims are intended to encompass the targeted modification methods that the Examiner indicated were enabled (see, pg. 7 of the Office Action). Support for these amendments is found throughout the specification, see for example, the claims as originally filed, as well as on pg. 4, [00020]; pg. 10, [00035]; and pg. 11, [00037]. No new matter is added. Applicants submit that in view of the above amendments, the enablement rejections are now moot.

Claim Rejections - 35 USC §103

Claims 1-4, 7-10, 12 and 13 are rejected under 35 USC §103(a) as unpatentable over Benvenisty et al. (US 2002/0127715 or WO 02/061033). Specifically, the Examiner asserts that Benvenisty teaches electroporation of genetic constructs into human embryonic stem (ES) cells to transform cells with a construction comprising a marker for identification and purification of transformed cells. The Examiner also asserts Benvenisty does not teach (1) integration of the vector by homologous recombination or (2) insertion of the marker into

regulatory regions of the genome such that its expression is regulated according to its state of differentiation. Applicants traverse the rejection.

In responding to this rejection, it is noted that the differences between Benvenisty et al. and the claimed invention were contemplated and described in the background section of the present application (see pg. 2, [0005]). Applicants' specification provides that, Benvenisty et al. does not disclose altering the genetics or the expression of native human genes in human embryonic stem (ES) cells. Benvenisty et al. uses expression vectors to randomly insert exogenous genes into cells. Benvenisty does not disclose controlled targeted gene replacement or "swapping" of native human genes, normally expressed in human ES cell culture, via homologous recombination and electroporation (see for example, Col. 1, [0008]). Methods used to obtain a sufficiently high enough level of transfection and/or integration in mammalian cells to practice the claimed invention were simply not defined by Benvenisty or any other document until applicants' filing. Although the desirability may have been there to achieve targeted integration, the know-how was not!

In fact, Benvenisty et al. surveys a variety of techniques for transfection of ES cells and concludes that the most abundant gene expression was achieved using a transfection method based on cationic polymers, including polymers of ethyleneimine, not electroporation (see Fig. 1 of Benvenisty et al.). In stark contrast, applicants' employ a modified electroporation technique to transfect human ES cells with foreign DNA at reasonable efficiencies to enable the claimed embodiments. This step was important for ensuring that the targeted gene modification worked in human ES cells. Before the applicants, no one succeeded in employing electroporation along with homologous recombination to achieve controlled targeted gene insertion in human ES cells. Further, applicants' electroporation step is a non-obvious modification of previously disclosed murine techniques.

Indeed, there are several research articles which discuss the infrequency and the problems associated with homologous recombination in mammalian cells, needless to say the technical hurdles were even harder to overcome for human ES cells. (See for example, Vasquez et al. "Manipulating the Mammalian Genome by Homologous recombination," *PNAS* 98:15, 8403-8410 (2001), citing at least two limitations constraining gene targeting in mammalian cells). Further, Benvenisty recites that "any known method for inserting, deleting or modifying a desired gene from a mammalian cell combined with transfection techniques

described in Examples 1-5 can be employed." (See Benvenisty at page 10, Col. 1). Benvenisty goes on to list patents that allegedly effect gene knock-outs. However, Benvenisty does not disclose human ES cells with genes knocked out. Furthermore, the patents relied on by Benvenisty and thus, Benvenisty itself, are non-enabling with respect to human ES cells. At best the cited patents disclose methods for manipulating mouse stem cells and it is well known that methods used on mouse ES cells do not work on human ES cells.

Human ES cells fundamentally and organically behave differently from mouse ES cells. In relation to the claimed embodiments, applicants discovered that for example, among other distinguishing variables, in mice genetic manipulation could be done on single cells. However, human ES cells are more social and needed to be in "clumps" for the electroporation technique to work effectively (e.g. with high efficiency) in humans. Other factors also had to be modified for the claimed method to work in human ES cells.

There are numerous examples in the literature where methods for effectively culturing and differentiating mouse ES cells do not apply equally well or at all to human ES cells. For example, conditions which sustain mouse ES cell cultures and support mouse ES cell differentiation do not apply equally to human ES cell cultures. Mouse, but not human, ES cells require LIF in the culture media to remain undifferentiated. Human ES cells have no LIF receptor.

Mouse ES cells, but not human ES cells, have the SSEA-1 marker. Also, ES cells from mice and humans require distinct sets of factors to remain undifferentiated. For instance, the leukemia inhibitory factor (LIF)/Stat3 pathway, a key to mouse ES cell proliferation, does not support human ES cell proliferation, and appears inactive in conditions that support human ES cells (Daheron L, et al., LIF/STAT3 signaling fails to maintain self-renewal of human embryonic stem cells, *Stem Cells* (2004) 22:770-778).

Similarly, bone morphogenetic proteins (BMPs) together with LIF support mouse ESC self-renewal at clonal densities in serum-free medium (Ying QL, et al., *Cell* (2003) 115:281-292). However, when BMPs are added to mouse ES cells, they do not differentiate into trophoblast cells. On the contrary, BMPs cause rapid human ES cell differentiation to trophoblast cells in conditions that would otherwise support self-renewal, such as culture on fibroblasts or in fibroblast-conditioned medium (see, Xu RH, et al., *Nat Biotechnol* (2002)

20:1261-1264). Also, FGF signaling is important to self-renewal of ES cells from humans, but not from mice (Xu RH, et al. Basic FGF and suppression of BMP signaling sustain undifferentiated proliferation of human ESCs. *Nat Methods* (2005) 2:185-190). Clearly, the methods for manipulating (culturing and differentiating) mouse and human ES cells are not interchangeable. Thus, Benvenisty cannot be used alone or in combination with other documents to render the claimed embodiments obvious.

In an attempt to cure the deficiencies of Benvenisty, the Examiner cites West et al. (U.S. Pub. No. 20040219563). In response, applicants submit that West et al. is not an appropriately cited document, as it was filed after the priority date of the present application. Applicants' priority date is February 7, 2003, whereas the earliest filing date for West et al. is October 16, 2003. Applicants filed their application nearly 8 months before West et al. Therefore, West et al. cannot be relied upon to support a *prima facie* obviousness rejection.

In addition, like Benvenisty's disclosure, West et al. discloses only random integrants. West et al. discloses a method for determining the relative timing of the transcriptional activation or repression of genes in a population of murine and monkey stem cells by randomly inserting into the genomic DNA of stem cells a marker DNA construct. In contrast, the claimed embodiments require targeted genetic modifications of human ES cells through a combination of homologous recombination and electroporation to precisely insert a genetic construct into a selected site in the genome of human ES cells. Thus, applicants submit that it was not within the knowledge of one skilled in the art to simply combine the disclosures of Benvenisty and West to arrive at the claimed embodiments.

As further evidence of non-obviousness, applicants submit that even after the present application was filed, there was surprise and excitement within the stem cell research community because many had been trying to accomplish what the applicants successfully achieved. To support this assertion, applicants submit copies of online news articles from (1) the *Milwaukee Journal Sentinel* (MJS) from February 9, 2003 entitled "Scientists Swap Genes in Human Stem Cells" and (2) *Genomics & Genetics Weekly* (G&GW) from March 7, 2003, entitled "UW Scientists Swap Genes in Human Stem Cells" disclosed herewith in a supplemental information disclosure statement.

These articles include statements from leading stem cells scientists, such as George Daley of Mass. Institute of Technology, who are at "directly competing" research institutions

across the U.S. All of the scientists quoted in the articles were trying to do the same thing in their labs as the inventors successfully achieved. The quoted researchers all agree that this invention is a critical tool for doing gene and stem cell therapy (see for example, George Daley's quote at pg. 2 of MJS and pg. 14 bold text in G&GW). *More importantly, to support non-obviousness, the articles highlight the difficulty that stem cell researchers faced (up until the invention) in extending what was known in mice to humans.*

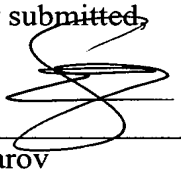
Applicants submit that at best, one skilled in the art might have found it obvious to combine the disclosures of Benvenisty and West to obtain targeted modifications in *human* ES cells. However, "obvious to try" is not the standard of 35 U.S.C. §103. As such, it is believed that a *prima facie* case of obviousness for the claimed embodiments cannot be made.

Accordingly, applicants respectfully request that in view of these claim amendments and remarks, the rejection be respectfully reconsidered, withdrawn and that a timely Notice of Allowance be issued in this case.

Fees

An extension of time is included so this response will be considered as timely filed. Please charge the fee to Deposit Account No. 17-0055. No other fees are believed to be due, but should any fees be required in this or any subsequent response, please consider this to be a request to charge the fees due to the same Deposit Account.

Respectfully submitted,



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